Electronic Supplementary Material (ESI) for Molecular BioSystems.

Epithelial-to-mesenchymal transition involves triacylglycerol accumulation in DU145 prostate cancer cells

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Supplementary material

EMT assessment by flow cytometry

EMT induction was confirmed under TNFα exposure in DU145 prostate cancer cells. EMT was assessed by flow cytometry on cells exposed to TNFα (20 ng/ml) for 24 hours, using a specific antibody against E-cadherin, a characteristic epithelial cell marker. The results showed a reduction of the fluorescence associated to membrane E-cadherin (Fig. S1), indicating that the reported conditions induced EMT in DU145 cells.

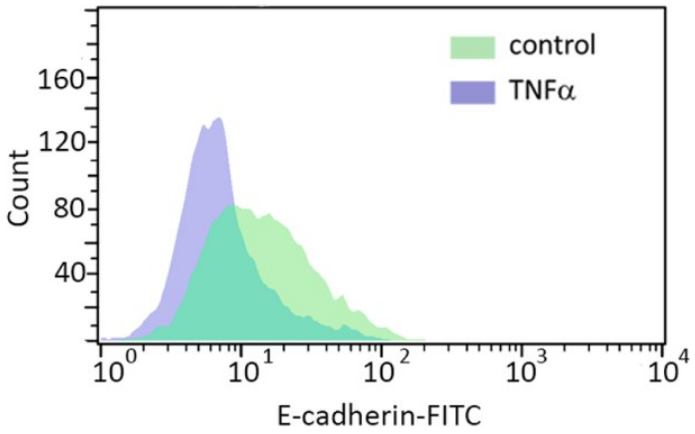

Fig S1. EMT induction in DU145 prostate cancer cells under TNFα treatment. Histogram of E-cadherin fluorescence intensity (FITC). DU145 prostate cancer cells were treated for 24 h with TNFα (20ng/ml) and stained with the anti E-cadherin-FITC antibody for 1 hour. The image illustrates a representative histogram of three independent measurements.

Exploratory analysis of TICs

A preliminary study on total ion current (TIC) chromatograms, obtained in both positive and negative ionization modes was carried out by Partial Least Squares-Discriminant Analysis (PLS-DA). This classification study was performed on matrices containing the normalized TICs of samples, in

order to see if their lipidomic profiles could separate samples corresponding to different times of TNFα exposure. PLS-DA is a supervised classification analysis tool that requires prior knowledge of class membership. In this case, two classes were chosen and defined as pre-EMT and post-EMT: samples at 0 and 3 hours were grouped into the pre-EMT class and samples at 5 and 6 hours into the post-EMT class. These classes were defined considering that at 0 and 3 hours mRNA levels of the EMT markers were similar and significantly different to the expression levels observed at 5 and 6 hours. As shown in the scores plot of the PLS-DA model (Fig. S2), the use of this class assignment resulted in a good discrimination between pre-EMT and post-EMT classes for both extraction 1 and 2 samples in the positive ionization mode. Samples at 4 hour-treatment were left out of the calculations as its inclusion in either pre or post-EMT classes impeded the correct separation of samples, probably due to an intermediate lipidic profile between pre and post-EMT samples. In contrast, the TICs of samples acquired in the negative mode in both extraction 1 and 2 could not be separated in the PLS-DA models, thus these data was not considered for further analysis. The clear separation between classes considering the TIC chromatograms in the positive mode suggested that substantial changes in the lipidomic profile occurred as a consequence of the EMT induction under TNFα treatment.

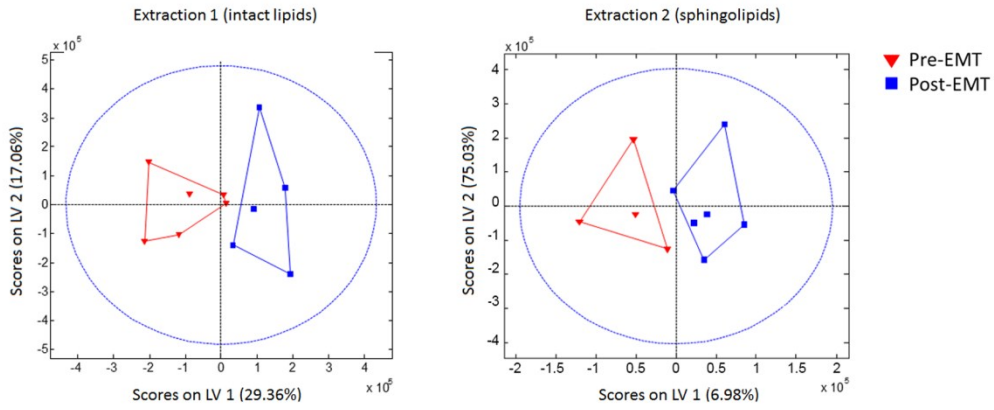

Figure S2. PLS-DA analysis of lipidomic data. Lipid extracts of DU145 cells treated with TNFα at different times were analysed through UPLC-TOF and data was imported into MATLAB environment. PLS-DA scores plots of matrices containing TICs data in the positive ionization mode of both types of extractions. Two classes were defined: pre-EMT, containing samples of 0 and 3h treatment and post-EMT, with the samples of 5 and 6h.

LC-MS profiles of samples and internal standards

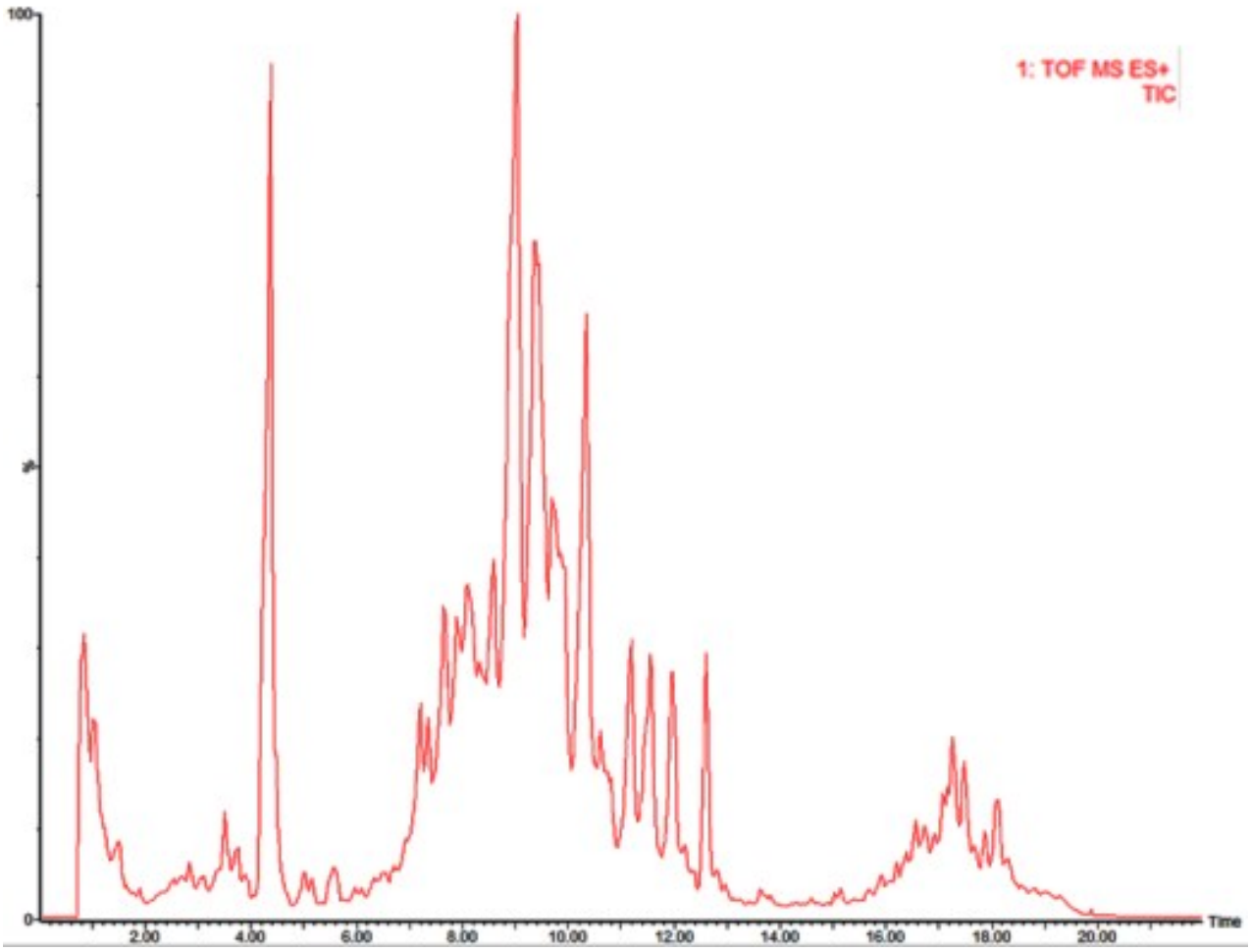

Figure S3. Representative LC-MS profile of extraction 1 samples

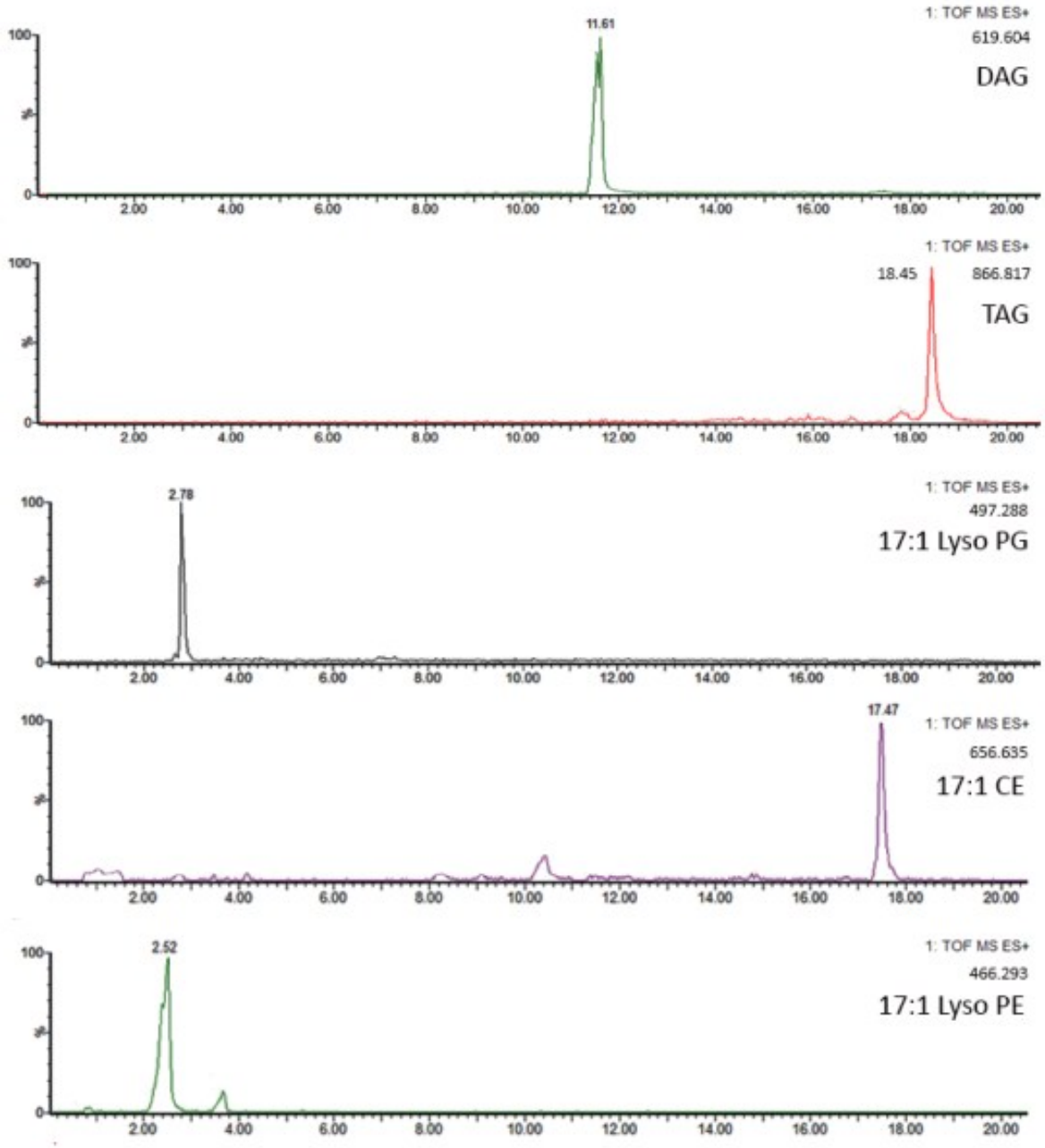

Figure S4. LC-MS profiles of internal standards added in extraction 1. DAG: 1,3-17:0 D5 diacylgliceride; TAG: 1,2,3-17:0 triacylglyceride; 17:1 Lyso PG: 17:1 lyso phosphoglyceride; 17:0 CE: 17:0 cholesteryl ester; 17:1 lysoPE: 17:1 lyso phosphatidylethanolamine.

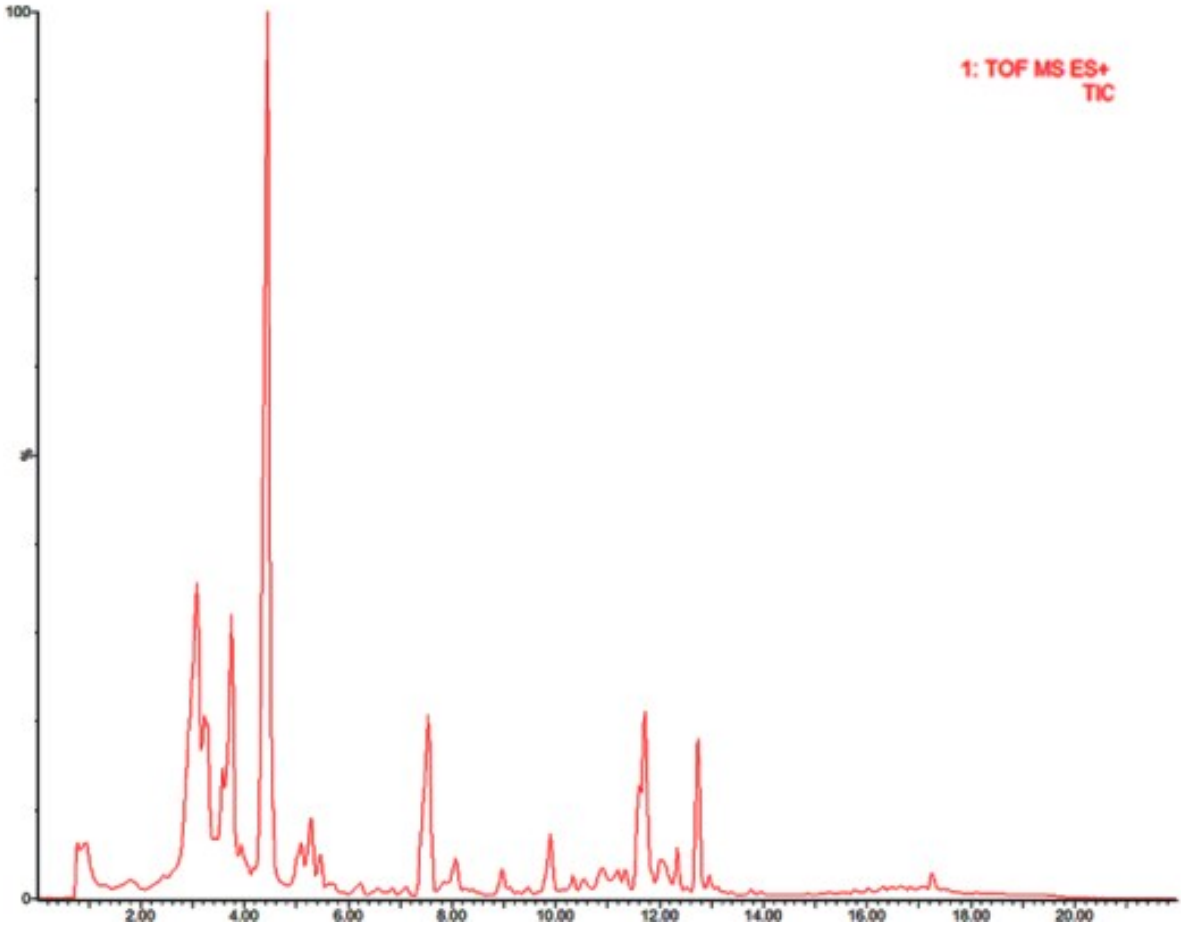

Figure S5. Representative LC-MS profile of extraction 2 samples

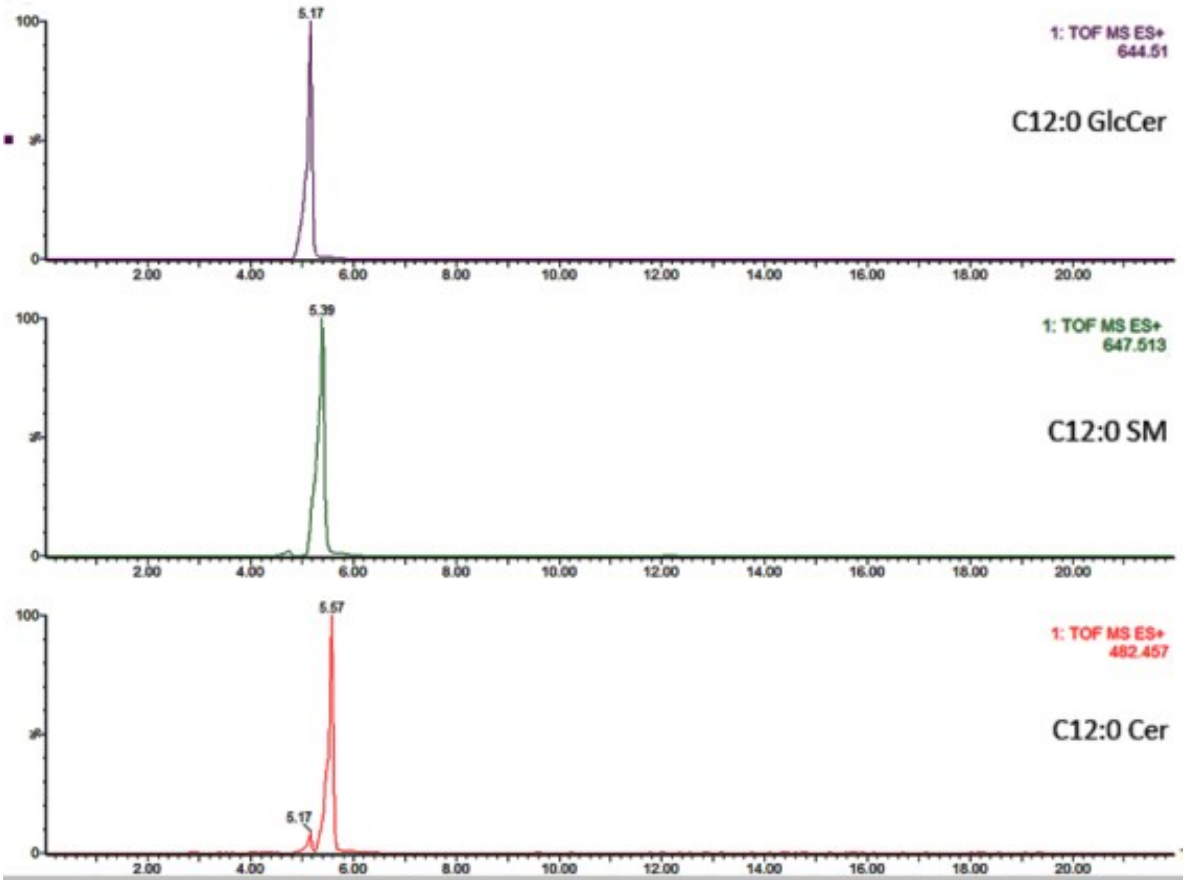

Figure S6. LC-MS profiles of internal standards added in extraction 2. C12:0 GlcCer: C12 glucosylceramide or N-dodecanoylglucosyl-sphingosine; C12:0 SM: C12 sphingomyelin or N-dodecanoylsphingosylphosphorylcholine; C12:0 Cer: C12 ceramide or N-dodecanoylsphingosine.

Experimental

Flow Cytometry Analysis

The EMT assessment by flow cytometry was performed following the recommendations previously reported by Strauss et al.[1] Briefly, cells were plated at a density of 2x105 cells per well in 12-well plates and left in culture until next day. Cells were treated with 20 ng/ml TNFα for 24 hours. Cells were harvested using PBS/EDTA for 5 minutes to preserve the E-cadherin in the cell membrane. Cells were washed twice, and cell pellets were stained with 20 µl/sample of anti CD324/E-cadherin conjugated to FITC (Life Technologies), according to the manufacturer's instructions. Samples were incubated on ice for one hour, and then washed with 200 µl of PBS. Green fluorescence emission of 5000 cells was measured for each sample with a Guava flow cytometer (Millipore), using Incyte software (Millipore). Calculated values of mean green fluorescence of histograms were used to compare TNF treated and untreated cells.

Exploratory analysis

For the preliminary analysis on TICS, mass values at each retention time were summed to obtain the TIC of each sample. Then, two matrices containing the TICs of treated samples at different times were constructed in both positive and negative ionization modes. Matrices were baseline corrected using the asymmetric least squares algorithm 2 and mean-centered. Then, the matrices were analysed by PLS-DA using the PLS Toolbox software for MATLAB. Data was cross-validated by applying the leave-one-out method, which was adequate for the small number of samples in this study. Three out of the twenty-four samples analysed were removed from the study as they appeared as outliers in the Q residuals *vs* T^2 plots. Taking into account the correct separation between classes and the results of qRT-PCR, pre-EMT class was defined by 0 and 3h samples and post-EMT class by 5 and 6h samples.

References

1. R. Strauss, J. Bartek and A. Lieber, *Methods in molecular biology*, 2013, **1049**, 355-368.
2. P. H. Eilers, *Analytical chemistry*, 2003, **75**, 3631-3636.